Research Article

Antiacetylcholinesterase and Cytotoxic Activities of Egyptian Propolis with Correlation to its GC/MS and HPLC Analysis

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ABSTRACT

Acetylcholinesterase (AChE) inhibitors from natural resources are gaining an interest as new approach to treat the cognitive symptoms of Alzheimer disease (AD) and they can decrease the oxidative stress which directly related to neurodegenerative diseases and can protect tissues from the DNA-damaging effects. GC/MS analyses of propolis samples, revealed the identification of 74 compounds. Sample A: has eight caffeic acid esters (14.8%), where 3-Methyl-3-butenyl-*trans*-caffeate (7.29%) and 3-Methyl-2-butenyl- *trans*-caffeate (6.22%) represent the major characteristic esters, flavonoids (4.58%) where; Pinocembrin, Pinobanksin acetate, Chrysin and Galangin are majors. In B, aliphatic acids (9.98%), Phenolic compounds (2.65%), where 1,3-dihydroxy-5-heptadecenylbenzene and other two derivatives (C17:0, C19:1) are found only in sample B (as new compounds to propolis), Triterpenoids (3.23%); lanosterol, β-amyrin, cycloartanol are present only in sample B, beside new triterpenoids. In HPLC analyses; chrysin-7-methylether, Chrysin, quercetin-3,7-dimethylether, pinocembrin, genistein and Phenylethyl-caffeate were significantly present in high concentrations in sample A. The two samples showed strong DPPH radical activity at 25 µg/ ml. IC_{50} was determined as 4.69 µg/ml for sample A and 5.1 µg/ml for sample B. As acetylcholinesterase inhibitor; A showed stronger inhibitory activity than B. IC_{50} was determined as 360 µg/ml for sample A and more than 600 µg/ml for sample B. Sample A showed the highest cytotoxic effect on HELA (Cervical) cells with an IC_{50} 10.1µg/ml. Both A and B show moderate cytotoxic activity against HEPG2 (Liver) carcinoma cell line. Conclusion, it is the first study that assesses the effect of Egyptian propolis on AChE and cytotoxic effect on HELA (Cervical) cells.

Keywords: Propolis, Acetylcholinesterase inhibitors, Cytotoxic activity, Chemical Composition; GC/MS and HPLC analysis

INTRODUCTION

The aging process correlates with a progressive failure in the normal cellular and organ functioning; these alterations are aggravated in Alzheimer's disease (AD). In both aging and AD there is a general decrease in the capacity of the body to eliminate toxic compounds and supply the brain with relevant growth and nutritional factors.[1] One important change observed in the brain of AD patients is a decrease in level of the neurotransmitter acetylcholine (ACh) by nearly 90%, which affects behavioral aspects and causes impairment in cognitive function.[2]

In the recent decades, there is an increasing interest in finding naturally occurring antioxidants for medicinal applications,[3] the acetylcholinesterase enzyme (AChE) is an attractive target for drug design and for the discovery of mechanism-based inhibitors because of its role in the hydrolysis of the neurotransmitter (ACh). AChE inhibitors are the most effective approach to treat the cognitive symptoms of Alzheimer disease (AD). Oxidative stress is directly related to neurodegenerative diseases; therefore, the antioxidant potentials of various natural products extracts can be helpful to provide neuroprotection.[4]

Propolis from various geographical locations, bee species and seasons,[5] as well as their different extracts, have been reported to exhibit a diverse array of bioactivities, such as antibacterial,[6] antifungal,[7] antiparasitic,[8] antioxidant and inhibition of LDL peroxidation,[9] anti-inflammatory[10] and antiproliferative/cytotoxic activities.[11] The main chemical classes found in propolis, which appear to be the principal components responsible for the biological activities of propolis samples, include flavonoids, aromatic acids, diterpenic acids and phenolic compounds.[12]

The polyphenolic/flavonoids concentrated in propolis are powerful antioxidants that can protect tissues from the DNA-damaging effects of a variety of harmful chemicals and prevent cancer by scavenging oxidizing species.[13] Furthermore the use of propolis as an immuno-stimulatory adjuvant for treatment of tumours is slowly gaining ground. Pre-clinical studies showed that co-administration of a propolis extract together with traditional chemotherapy resulted in better regression of tumours.[14]

Due to these supposed beneficial effects, there is a renewal of interest in the composition and biological activities of propolis.

Till now to our knowledge; there is no data about Egyptian propolis and its effect on AChE enzyme although there are plenty of data concernant the individual compounds and their effect on the enzyme.

This finding prompted us for further investigations on the Egyptian propolis and whether it has a role in alleviating

AD symptoms through the inhibition of the enzyme AChE activity or not.

So, the aim of the present work was to evaluate the anticholinesterase, cytotoxic and antioxidant activities of two propolis samples from different localities with the correlative studies of their chemical composition with GC/MS and HPLC analysis.

MATERIALS AND METHODS

Propolis

Two Egyptian propolis samples were collected from two different provinces (propolis sample A and propolis B) in April, 2014 and stored at –20 °C until investigation.

Extraction and sample preparation

Propolis samples were extracted at room temperature with 50 ml of 70% ethanol (twice after 24 h). The alcoholic extract was evaporated at 50 °C until dryness. The percentage of extracted matter was as follows: Propolis A; 0.65 g/dry weight, and Propolis B; 0.41 g/dry weight. 1.5 mg of the dried matter was prepared for chromatography by derivatization for 30 min at 80 °C with 20 µl pyridine + 30 µl N,O, bis-(trimethylsilyl) trifluoroacetamide (BSTFA) and analyzed by GC/MS.[15]

GC/MS Analyses

A Finnigan MAT SSQ 7000 mass spectrometer was coupled with a Varian 3400 gas chromatograph. DB-1 column, 30 m x 0.32 mm (internal diameter) , was employed with helium as carrier gas (He pressure, 20 Mpa/cm^2), injector temperature, 310°C; GC temperature program, 85 - 310°C at 3 °C/ min (10 min. initial hold).The mass spectra were recorded in electron ionization (EI) mode at 70 eV. The scan repetition rate was 0.5 s over a mass range of 39 - 650 atomic mass units (amu).

Identification of compounds

The identification was accomplished using computer search user-generated reference libraries, incorporating mass spectra. Peaks were examined by single-ion chromatographic reconstruction to confirm their homogeneity. In some cases, when identical spectra have not been found, only the structural type of the corresponding component was proposed on the basis of its mass spectral fragmentation. Reference compounds were co-chromatographed when possible to confirm GC retention times.

HPLC analysis of propolis

Propolis extracts were dissolved in MeOH. Both the mobile phase and the dissolved materials were filtered by a Millex-HX Nylon syringe filter (0.45 um, 25 mm; Millipore, Bedford, MA). The materials are subjected to chromatographic analysis with High-Performance liquid Chromatography (HPLC), Reverse phase with the following specifications; Agilent1100 series liquid chromatograph: Quaternary pump (G1311A), Degasser (G1322A), Thermostatic Auto sampler (G1329A), Variable wavelength detector (G1314A) and column: phenomenex RP-18 (UK; 250 x 4.00 mm, 5 micron).

Elution was with water/formic acid (19:1 v/v; solvent A) and acetonitrile (solvent B), and the flow rate was 1 ml/min. Gradient elution started with 20% B, reaches 25% B at 25 min and 30% B at 35 min, and then the system became isocratic until 50 min, reaches 50% B at 60 min and 70% B at 67 min, at ambient temperature. The mobile phase solvents are HPLC grade and di-ionized H_2O.The compounds were detected with a UV detector and the chromatograms were recorded at 340 and 290 nm for flavones and flavanones, respectively.[16] Response factors for the authentic markers and the concentration of compounds in each propolis sample were calculated according to Ogan and Katz.[17]

DPPH radical scavenging activity

The two extracts were studied for DPPH radical scavenging activity following the modified procedure of Matsushige.[18] The absorbance was measured at 520 nm. All the reactions were performed in triplicate in 96-well micro-plate.

Acetylcholinesterase (AChE) inhibitory activity

The AChE-inhibitory activity was performed followed the method previously described,[19] with slight modification.[20] Electric-eel AChE (Sigma) was utilized; the enzymatic hydrolysis of acetylthiocholine was measured at a wavelength of 412 nm (15 min). All the reactions were performed in triplicate in 96-well micro-plate.

Evaluation of cytotoxic activity of A and B propolis samples

Human HELA (cervical) and HEPG2 (liver) cell lines were obtained from the American Type Culture Collection (ATCC, Minisota, U.S.A.). All cell lines were cultured in RPMI-1640 medium (Sigma Aldrich Chemical Co., St. Louis. Mo. U.S.A) supplemented with 10% FBS (Fetal bovine serum), penicillin (100 U/mL) and streptomycin (2mg/mL) at 5% CO_2 in a 37°C incubator. The cells were plated in 96-well plate at a density of 3.0x10^3 in 150µL of medium per well. Tested extracts dissolved in DMSO were added to the wells in triplicates with concentrations of 0, 5, 12.5, 25 and 50 µg/mL for 48h. The cytotoxic activity was determined using Sulphorhodamine-B (SRB) assay following the method reported by Vichai & Kirtikara,[21] theIC_{50} values were also calculated.

RESULTS AND DISCUSSION

Two propolis samples were collected from different provinces (Delta Egypt), each province characterized by the presence of predominant trees or shrubs. The samples were extracted at room temperature with 70% ethanol, the samples were analyzed with HPLC and silylated to subjected to GC/MS analysis for further analysis. The results obtained are summarized (Table 1-2, Figures1-3).

GC/MS analyses

It revealed the identification of 74 compounds; 45 for sample A and 58 for sample B. Aliphatic, phenolic acids and their esters, flavonoids, triterpenoids and other compounds were identified (Table 1, Figure 1-2).

Propolis sample A was characterized by the presence of:

- **Five phenolic acids**; 4-methoxy- , 4- hydroxy- , 3,4-dimethoxy- , 3,4-dihydroxy- , 4-hydroxy-3-methoxy-cinnamic acid derivatives were identified (Table 1).
- **Twelve phenolic acid esters** (14.99%) were identified, from which eight caffeic acid esters (14.8%). The major characteristic esters were 3-Methyl-3-butenyl-*trans*-caffeate (7.29%) and 3-Methyl-2-butenyl- *trans*-caffeate (6.22%)(Table 1, Figure 1), that is besides other esters like 3-Methyl-3-butenyl-*cis*-caffeate, 2-Methyl-butyl-*trans*-caffeate, 2-Methyl-2-butenyl-*trans*-caffeate, Benzyl caffeate, Phenylethyl caffeate, Decyl caffeate (a new compound to propolis) and the minor presence of Prenyl-isoferulate, Prenylferulate, Prenyl-*cis*-p-coumarate and Prenyl-*trans*-p-coumarate (Table 1).
- **Seven flavonoids** (4.58%) were identified; Pinocembrin, Pinobankasin acetate, Chrysin and Galangin are the major ones (Table 1, Figure 1).

Propolis sample B was characterized by the presence of:

- **Fifteen aliphatic acids** (9.98%) and five aliphatic acid **esters** (3.41%) were present, where hexadecanoic, octadecenoic acids and their ethyl esters are the majors (Table 1).
- **Phenolic compounds (2.65%), where 1,3-dihydroxy-5-heptadecenylbenzene** and other two derivatives (C17:0, C19:1) are found only in sample B (as new compounds to propolis), that is beside the presence of 4-hydroxyphenyl-ethanol and 4-phenyl-4-hydroxy-butanol (Table 1, Figure 2).
- **Triterpenoids (3.23%):** the known triterpenoids to propolis; lanosterol, β-amyrin, cycloartanol are present only in sample B (Table 1, Figure 2), beside the new triterpenoids 4,4-Dimethyl-3-oxacholest-5-en-7-one and 9,19-Cyclolanostan-3-ol-24-methylene, acetate; which were tentatively identified from mass spectra.

HPLC analysis of propolis

Twenty seven flavonoid compounds and two caffeic acid esters were quantitatively identified in propolis samples A and B. The flavone chrysin-7-methylether (119.7 mg), Chrysin (13.8 mg), the flavonol quercetin-3,7-dimethylether (75.9 mg), the flavanones pinocembrin (28.4 mg), the isoflavonegenistein (16.2 mg) and Phenylethyl-caffeate (CAPE)(66.6 mg/g propolis) were significantly present in high concentrations in sample A. The flavone chrysin-7-methylether (34 mg), the flavonol quercetin-3,7-dimethylether (48 mg), the flavanone pinostrobin (29.8 mg/g propolis), were found in moderate concentrations in sample B. The other flavonoids were found in very minor concentrations in both samples (Table 2, Figure 3).

The GC/MS comparable analysis (Table 1) showed that the major compound's groups assessed are; fatty acids (A, 1.7% and B, 9.98%), fatty acid esters (A, 0.03% and B, 3.41%), Phenolic compounds (A, 1.37% and B, 2.65%), Phenolic acid esters (A, 14.99% and B, 8.53%), flavonoids (A, 4.58% and B, 3.47%) and triterpenoids (A, 0.75% and B, 3.23%), while the HPLC comparable study revealed the identification of flavones (135 mg for sample A and 39 mg for sample B), flavonols (93 mg for A, 53 mg for B), flavanones (46.6mg A, 35.7mg B), isoflavones (18.9 mg only for A sample) and Caffeic acid esters (70 mg for A, 7mg/g propolis for B). our data are in agreements with previous studies.[8,22]

According to the difference in plant sources, each propolis sample characterized by certain specific compounds; the two propolis samples showed variations in their chemical composition and this could be attributed to the different localities they came from. Egyptian propolis showed strong variabilities due to the different geographic locations and plant origin, our results are in agreements with previous studies.[9,23-26]

DPPH radical scavenging activity

The DPPH radical scavenging activity was evaluated for propolis samples A and B in different concentrations. It was clear that the two samples showed strong DPPH radical activity at 25µg/ml (Figure 4). IC_{50} was determined as 4.69 µg/ml for sample A and 5.1 µg/ml for sample B. The DPPH assay is based on the principle that a hydrogen donor is an antioxidant. It measures the activity of an antioxidant to directly scavenge DPPH radical and determining its absorbance spectrophotometrically at 520 nm. The obtained data are in agreement with previous studies,[27-30] the strong DPPH radical scavenging activity of both samples is explained by their diverse chemical composition. From the GC/MS and HPLC data obtained, it could be concluded that the high content of flavonoids, CAPE, phenolic acids and their esters explain the high antioxidant activity. These data are in agreement with our previous work.[31-33]

Acetylcholinesterase (AChE) inhibitory activity

The inhibitory activity of two Egyptian propolis samples on the enzyme AChE was studied. At high concentrations (400-600 µg/ml), both extracts show strong inhibitory activity, although extract A showed stronger inhibitory activity than B. At low concentration (25-200 µg/ml) there was no activity detected; A showed a very very weak activity (5% at 200 µg/ml)(Figure 5).

IC_{50} was determined as 360µg/ml for sample A and more than 600µg/ml for sample B. From all the above mentioned data it could be concluded that the high activity of propolis sample A is due to its high content of

several classes of compounds that is known to possess high activity against the enzyme such as flavonoids, phenolic acids and their esters. While for sample B, its high content of the saturated aliphatic acids explains its much less activity than A; in our previous work, it was found that only the unsaturated aliphatic acids showed acetylcholinesterase inhibitory activity.[34,35]

The triterpenoids cycloartenol; previously isolated from Egyptian propolis was found to induce moderate inhibition to AChE with an IC_{50}value of 3.6±0.1 µM.[2] It was reported that Caffeic acid phenethyl ester (CAPE) inhibited the acetylcholinesterase activity in an *in-vitro* assay[36] and may increase the muscarinic-nicotinic hyperactivation.[37] Szwajgier and Borowiec studied ferulic, p-coumaric and caffeic acids. They concluded that p-coumaric acid had the largest share in the anticholinesterase activity.[38]

It was demonstrated that pinocembrin improves cognition and protects the neurovascular unit in Alzheimer related deficits.[39] Galangin was reported to show an inhibitory effect on AChE activity with the highest inhibition by over 55% and an IC_{50} of 120 µM.

The isolated flavonoids (pinostrobin, chrysin and 7-methoxychrysin) at the concentration of 10 µM have no inhibitory effect.[2] Orhana found that only quercetin showed a substantial inhibition (76.2%) against AChE, while genistein (65.7%) exerted a moderate inhibition on BChE.[41]

Min reported the flavonoids quercetin and kaempferol showed potential inhibitory activities against AChE with IC_{50} values of 25.9 and 30.4µM, respectively.[42] An important factor that may explain the high activity of sample A, besides its high content of flavonoids, is the structural aspects of flavonoids themselves. Xie found that the hydroxyl groups in the A ring of flavonoids is favorable for inhibiting AChE and that hydroxylation increases the affinities for the enzyme.[43]

Evaluation of cytotoxic activity of A and B propolis samples

In this research, propolis was used to determine the *in-vitro* antiproliferative/cytotoxic activity on two human cancer cell lines; HELA (cervical) and HEPG2 (liver). Both samples revealed a strong and broadly similar antiproliferative/cytotoxic activity on the two tested cell lines in a dose-dependent manner (Figure 6).

In terms of the antiproliferative/cytotoxic IC_{50} values; extract A showed the highest cytotoxic effect on HELA (Cervical) cells with an IC_{50} 10.1µg/ml and moderateIC_{50} in HEPG2 (Liver) carcinoma cell line (20.2µg/ml). Extract B showed moderate cytotoxic activity against both cell lines with an IC_{50} of 19.8 in both cell lines.

The further analysis of both samples with GC/MS and HPLC, revealed the presence of several bioactive compounds that are reported to display antiproliferative/cytotoxic activities. These compounds include phenolic acids, their esters, flavonoids and other compounds; these compounds did not only account for the net antiproliferation/cytotoxic activity of the crude extracts but also suggesting the existence of synergistic interactions in the propolis extracts.[44] From our previous studies it was proven that, the flavonoids pinostrobin and chrysin showed moderate anti-proliferative activity against HepG2 cell line (at 50 µM). Chrysin showed a significant activity (46 %) than that of pinostrobin, while cycloartinol had no activity,[2] our data are in agreement with several other studies. Propolis and its phenolic compounds have been reported to induce the death of cancer cells either by necrosis or by apoptosis, the latter of which might be by mitochondria mediated- or death signal mediated-apoptosis. (CAPE) currently seems to be a potential anti-cancer drug since it can inhibit the growth of many cell lines and it is only cytotoxic to cancer cell and not to normal cells *in-vitro*.[44] Quercetin, caffeic acid, caffeic acid phenethyl ester, galangin, genistein are the most promising of the antitumour agents.[45]

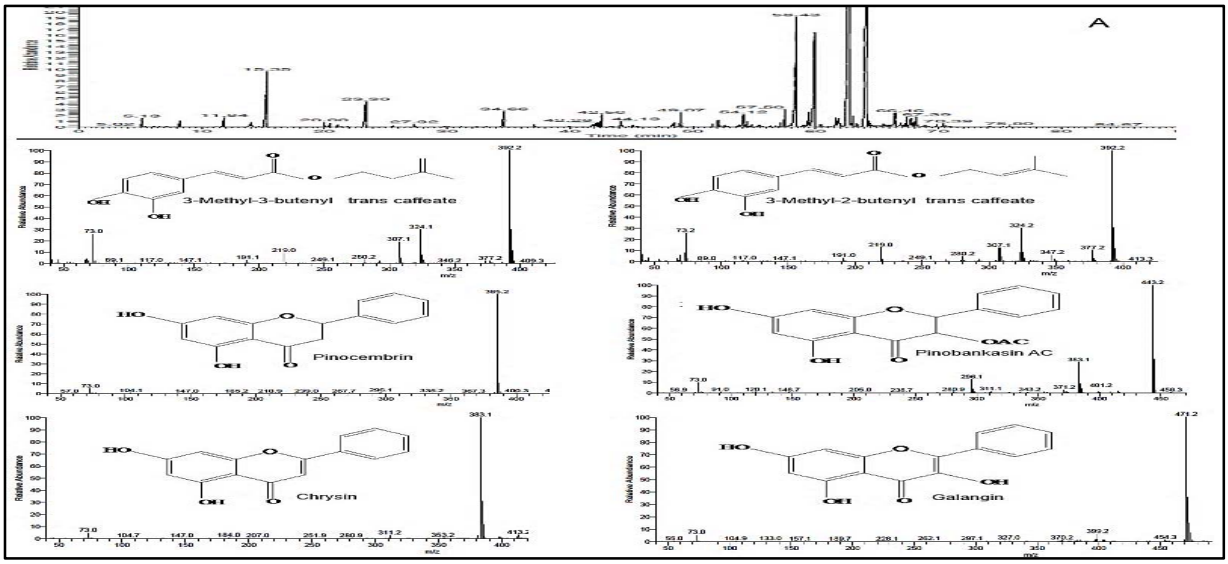

Figure 1: GC/MS Chromatogram of propolis sample (A) and the mass spectra of the prominent peaks.

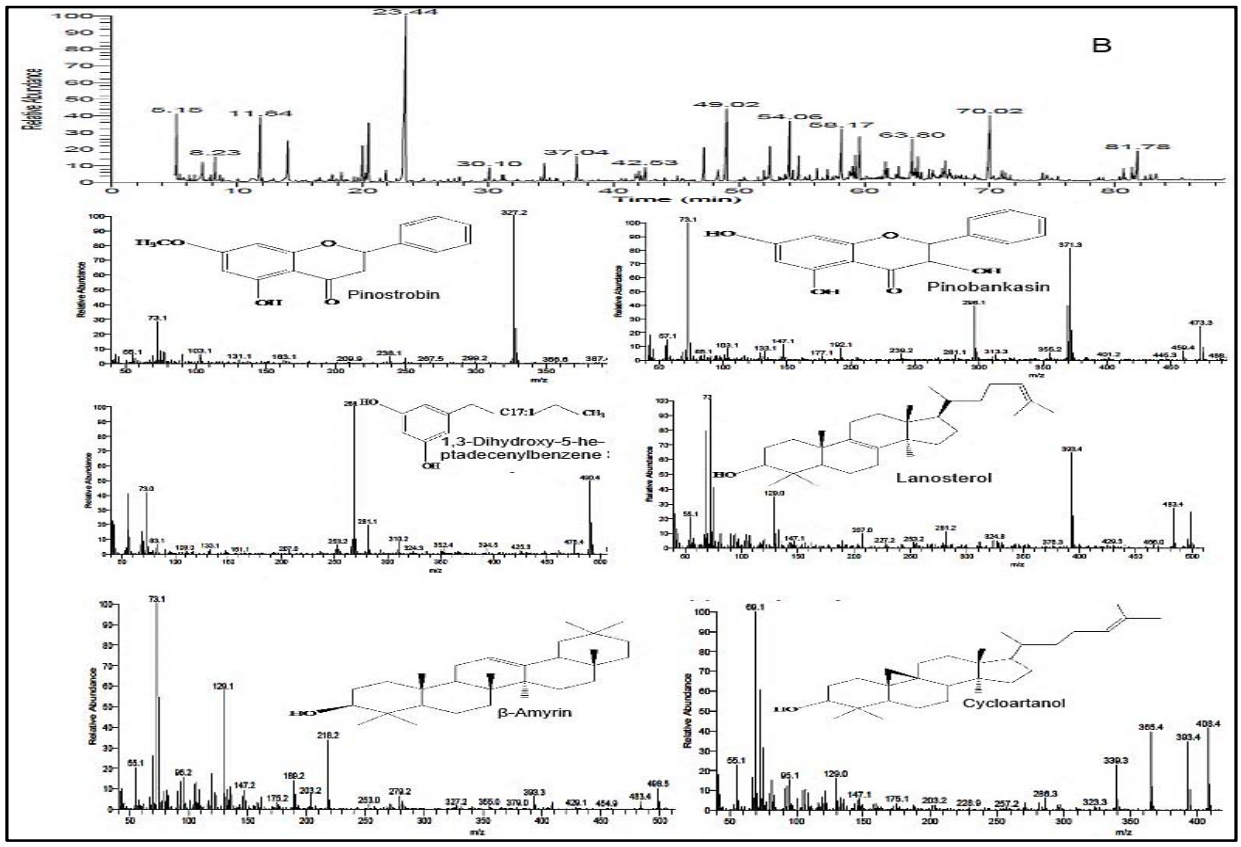

Figure 2: GC/MS Chromatogram of propolis sample (B) and the mass spectra of the prominent peaks

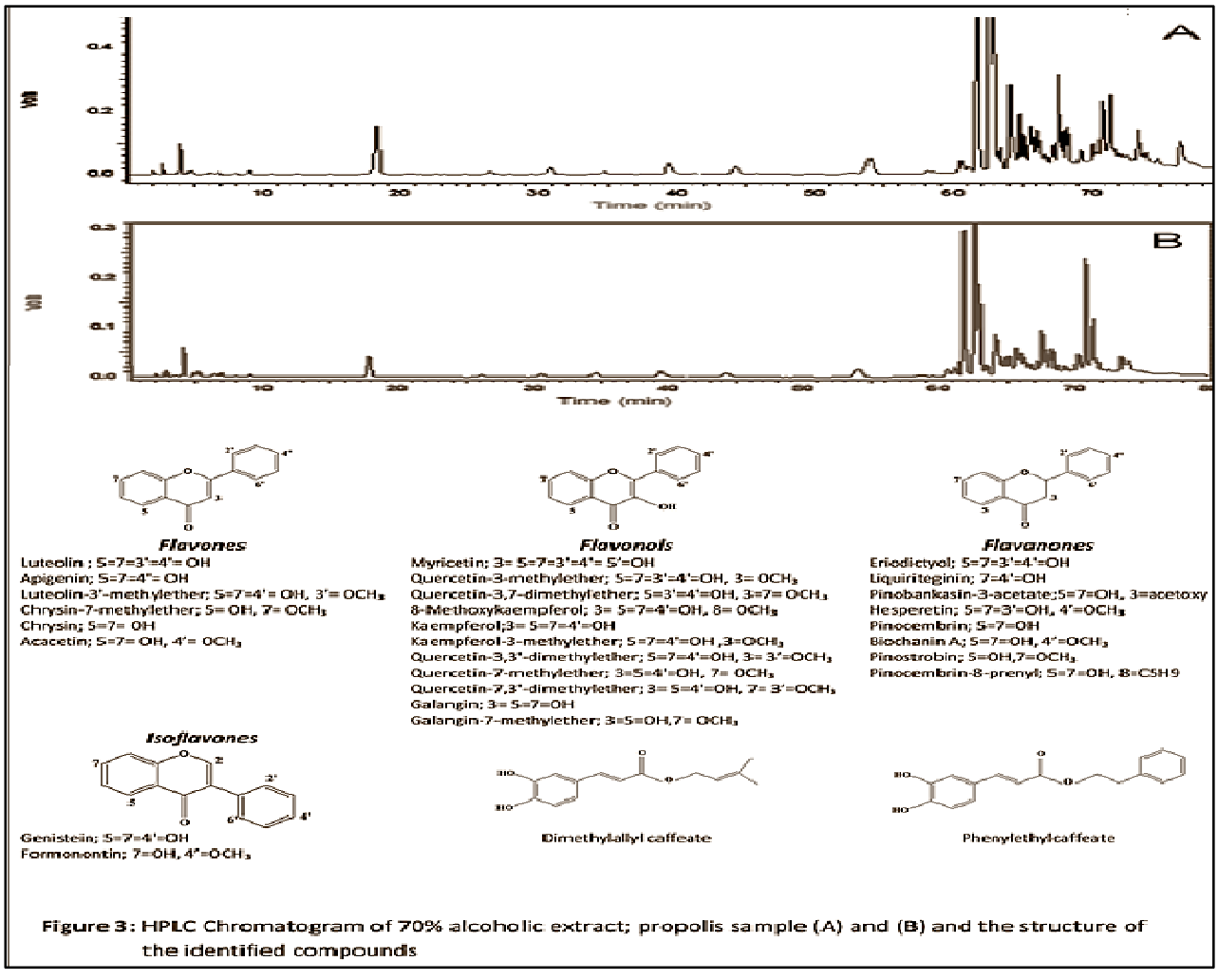

Figure 3: HPLC Chromatogram of 70% alcoholic extract; propolis sample (A) and (B) and the structure of the identified compounds

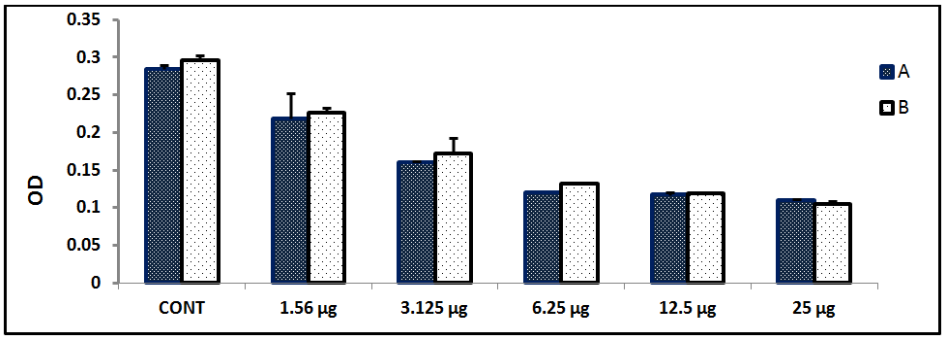

Figure 4: Free radical scavenging activity [**DPPH**] of propolis extracts; sample (A) and (B). Values are expressed as mean ± SD, n = 3 at different concentrations

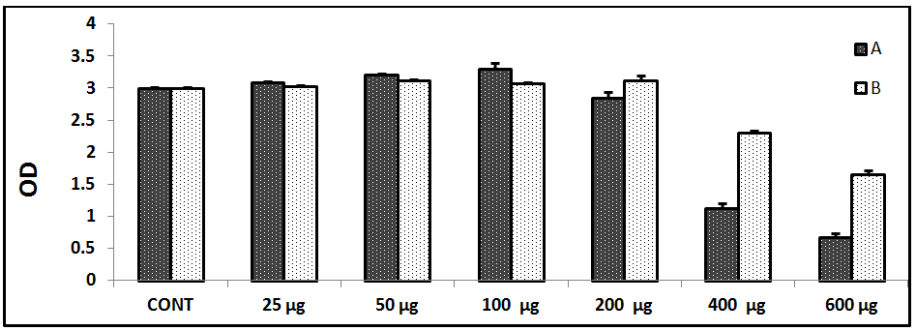

Figure 5: Inhibition of the **acetylcholinesterase** activity by propolis extracts; samples (A)and (B). Values are expressed as mean ± SD, n = 3 at different concentrations

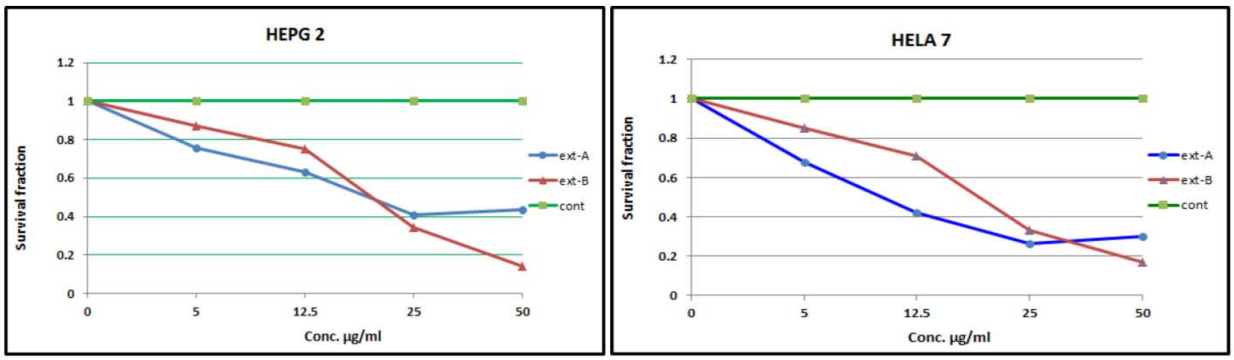

Figure 6: Effect of propolis extracts; sample (A) and (B) on HELA (Cervical) and HEPG2 (Liver) carcinoma cell lines viability.

Table 1: Chemical composition assessed by GC/MS of propolis alcoholic extract of samples (A) and (B)

No.	Compound	RT	*Propolis(A)	*Propolis(B)
	Aliphatic Acids			
1	2- hydroxyl-Propanoic acid	8.23	0.27	1.50
2	Hydroxyacetic acid	8.83	0.02	0.24
3	Propanoic acid, 2-oxo-3- hydroxy	9.43	0.02	
4	Ethanedioic acid,	12.01		0.32
5	Octanoic acid	18.68		0.11
6	Butanedioic acid,	21.41	0.04	
7	Propanoic acid, 2,3-dihydroxy	22.71	0.05	0.24
8	Decanoic acid,	27.71		0.20
9	2-Hydroxy-Butanedioic acid	29.81	0.06	0.27
10	2,3,4-Trihydroxybutyric acid	32.41	0.05	0.17
11	2,3,4-Trihydroxybutyric acid (isomer)	33.18	0.05	
12	3-Phenyl-3-hydroxypropanoic acid	33.55		0.09
13	Dodecanoic acid	35.45		0.09

14	Octanedioic acid	37.48		0.12
15	Azelaic acid,(C9- dioic acid)	41.03	0.01	0.14
16	Hexadecanoic acid	49.06	0.53	**3.18**
17	Octadecenoic acid	54.11	0.44	**2.29**
18	Octadecanoic acid	54.82	0.16	1.02
	Total		**1.7**	**9.98**
	Aliphatic Acid Esters			
19	Hexadecanoic acid ethyl ester	47.18	0.03	**1.87**
20	Octadecenoic acid ethyl ester	52.48		**0.93**
21	Octadecanoic acid ethyl ester	53.23		0.15
22	Hexadecanoic acid-15-hydroxy methyl ester	55.57		0.37
23	Ethyl tetracosanoate	68.77		0.09
	Total		**0.03**	**3.41**
	Phenolic compounds			
24	Benzene- 1,3-dihydroxy	17.93		0.22
25	Diphenyl ether	24.88		0.13
26	5-Hydroxy-6(1-hydroxyethyl) 2,7dimethoxy naphthoquinone	31.35		0.12
27	4-hydroxyphenyl ethanol	34.27	0.06	0.46
28	4-phenyl-4-hydroxy butanol	34.65	1.31	1.86
29	1[4-hydroxyphenyl]-3-[2,4-dihydroxyphenyl]-2-propen-1-one	62.53		0.26
30	1,3-dihydroxy-5-heptadecenylbenzene[new,]	71.23		0.32
31	1,3-dihydroxy-5-heptadecylbenzene[new,]	71.34		0.07
32	1,3-dihydroxy-5-nonadecenylbenzene[new,]	75.45		0.14
	Total		**1.37**	**2.65**
	Phenolic acids			
33	Benzoic acid	17.57		0.39
34	4-Methoxy cinnamic acid	41.89	0.1	0.06
35	4-Hydroxy cinnamic acid [Coumaric acid]	45.61	0.02	0.12
36	3,4-Dimethoxy-cinnamic acid	48.49	0.14	
37	4-Hydroxy-3-methoxy cinnamic acid [Ferulic acid]	50.04	0.02	
38	3,4-Dihydroxy-cinnamic acid [Caffeic acid]	52.06	0.48	0.79
	Total		**0.76**	**1.36**
	Phenolic acids Esters			
39	Prenyl-isoferulate	47.56	0.02	
40	Prenyl-*cis*-p-coumarate	52.23	0.04	0.09
41	3-Methyl-3-butenyl- *cis*- caffeate	53.55	0.03	
42	Prenyl-*trans*-p-coumarate	53.77	0.06	0.2
43	Prenylferulate	56.34	0.04	
44	2-Methylbutyl *trans* caffeate	57.91	0.15	0.15
45	3-Methyl-3-butenyl *trans* caffeate	58.41	**7.29**	2.93
46	2-Methyl-2-butenyl *trans* caffeate	59.47	0.87	1.47
47	3-Methyl-2-butenyl *trans* caffeate [Dimethylallyl caffeate]	59.92	**6.22**	2.63
48	Benzyl caffeate	67.16	0.17	0.27
49	Phenylethyl caffeate	69.02	0.08	
50	Decyl caffeate *[new]	70.97	0.02	
51	Tetradecenylcaffeate	74.54		0.24
52	Hexadecylcaffeate	74.64		0.35
	Total		**14.99**	**8.53**
	Flavonoids			
53	Pinostrobin	62.03	0.24	**1.07**

54	Pinocembrin	63.03	**1.14**	0.74
55	Pinobanksin	64.37	0.22	**1.07**
56	Pinobanksin acetate	66.46	**0.94**	
57	Chrysin	67.75	**0.83**	0.27
58	Galangin	68.18	**0.92**	0.32
59	Myricetin	70.39	**0.29**	---
	Total		**4.58**	**3.47**
	Terpenes			
60	Menthol	18.31		0.54
61	8-Azabicyclo[3.2.1]octane, 8-methyl-3-hydroxy	21.09	0.08	
62	Dehydroabietic acid	58.63		0.07
	Total		**0.08**	**0.61**
	Triterpenoids			
63	4,4-Dimethyl-3-oxacholest-5-en-7-one[new, t]	61.85	0.75	**1.14**
64	Lanosterol	80.35	----	**0.12**
65	β-Amyrin	80.66	----	**0.48**
66	Cycloartanol	81.78	----	**1.34**
67	9,19-Cyclolanostan-3-ol-24-methylene,acetate[new, t]	82.82	----	**0.15**
	Total		**0.75**	**3.23**
	others			
69	Alanine	27.32	0.15	
70	Phosphoric acid	40.64		0.22
71	2-Ketolgluconic acid,	41.49	0.03	
72	Quinic acid	44.13	0.29	0.2
73	Docosane	53.39		0.14
74	1-O-Octadecylglycerol	66.46		0.64
	Total		**0.47**	**1.2**

RT=retention time. * , TIC = The ion current generated depends on the characteristics of the compound concerned and it is not a true quantitation. [t],Tentatively identified by analysis of mass spectrum.

Table 2: Flavonoids assessed by HPLC of Egyptian propolis collected from different province (conc. mg / g propolis)

No.	Name	Chemical name	RT	propolis (A)	propolis (B)
		Flavones			
1	Luteolin	5,7,3',4'-tetrahydroxyflavone	24.53	0.05	0.01
2	Apigenin	5,7,4'-trihydroxyflavone	37.95		0.01
3	Luteolin-3'-methylether	5,7,4'-trihydroxy-3'-methoxyflavone	42.06	0.12	0.08
4	Chrysin-7-methylether	5- hydroxy-7-methoxy flavone	61.91	**119.7**	34.06
5	Chrysin	5,7-dihydroxyflavone	64.18	**13.8**	4.32
6	Acacetin	5,7- dihydroxy-4'-methoxy flavone	65.4	1.25	0.52
	Total			**134.9**	**39.0**
		Flavonols			
7	Myricetin	3,5,7,3',4',5'- hexahydroxyflavone	12.88	1.2	0.01
8	Quercetin-3-methylether	5,7,3',4'-tetrahydroxy-3-methoxyflavone	29.33	0.08	0.01
9	Quercetin-3,7-dimethylether	5,3',4'-trihydroxy-3,7-dimethoxyflavone	34.6	75.9	48.44
10	8-Methoxykaempferol	3,5,7,4'- tetrahydroxy-8- methoxyflavone	37.58	0.90	0.02
11	Kaempferol	3,5,7,4'- tetrahydroxyflavone	41.32	0.30	0.06
12	Kaempferol-3-methylether	5,7,4'- trihydroxy-3-methoxyflavone	44.46	1.01	0.32
13	Quercetin-3,3'-dimethylether	5,7,4'-trihydroxy-3,3'-dimethoxyflavone	45.53	0.10	0.04
14	Quercetin-7-methylether	3,5,3',4'-tetrahydroxy-7-methoxyflavone	56.88	0.03	---
15	Galangin	3,5,7- trihydroxyflavone	64.83	3.34	0.82
16	Quercetin-7,3'-dimethylether	3,5,4'-trihydroxy-7,3'-dimethoxyflavone	66.13	5.65	2.04

17	Galangin-7-methylether	3,5- dihydroxy-7-methoxy flavone	72.48	4.90	1.27
	Total			**93.41**	**53.03**
		Flavanones			
18	Eriodictyol	5,7,3',4'- tetrahydroxyflavanone	19.88	0.09	0.01
19	Liquiriteginin	7,4'-dihydroxyflavanone	20.45	0.55	0.02
20	Pinobanksin-3-acetate	5,7-dihydroxy-3-acetyloxyflavanone	33.26	0.74	---
21	Hesperetin	5,7,3'- trihydroxy-4'-methoxyflavanone	39.1	1.49	0.44
22	Pinocembrin	5,7-dihydroxyflavanone	62.73	28.40	4.61
23	Biochanin A	5,7-dihydroxy-4'-methoxyflavanone	65.15	2.21	0.03
24	Pinostrobin	5-hydroxy-7-methoxyflavanone	71.25	12.20	29.80
25	Pinocembrin-8-prenyl	5,7-dihydroxy-8-prenyl-flavanone	73.55	0.94	0.81
	Total			**46.62**	**35.72**
		Isoflavones			
26	Genistein	5,7,4'-trihydroxyisoflavone	35.8	16.16	---
27	Formonontin	7-hydroxy-4'-methoxyisoflavone	51.45	2.70	---
	Total			**18.86**	---
		Caffeic acid esters			
28	Dimethylallylcaffeate	3-methylbut-2-enyl caffeate	62.65	3.90	0.51
29	Phenylethylcaffeate	Phenylethyl-trans-caffeate	64.7	66.60	6.80
	Total			**70.5**	**7.31**

CONCLUSION

This is the first study that assesses the effect of Egyptian propolis on AChE. It could be concluded that, propolis sample A showed significant acetylcholinesterase inhibition, high cytotoxic effect on HELA (Cervical) cells with an IC_{50} 10.1µg/ml beside its high antioxidant activity.

Our study provides (for the first time) primary evidence suggesting propolis A in further *in-vivo* studies could play an important role as acetylcholinesterase inhibitor, antiproliferative and antioxidant activities.

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